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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Deb K. Chatterjee

Appln. No.: 09/558,421

Filed: April 26, 2000

For: **Mutant DNA Polymeràses and Uses Thereof**

Art Unit: 1652

Examiner: Rao, M.

Atty Docket: 0942.3600003/RWE/BJD

Showing by Applicants Under 37 C.F.R. § 1.608(b)

Commissioner for Patents
Washington, DC 20231

Sir:

Pursuant to the provisions of 37 C.F.R. § 1.607 and 1.643, Invitrogen Corporation, assignee of record of the entire interest of the present application, has filed concurrently herewith a Request for Interference Under 37 C.F.R. § 1.607, requesting the declaration of an interference between the present application (U.S. Appl. No. 09/558,421; hereinafter "the '421 application") and U.S. Patent No. 5,614,365 (hereinafter "the '365 patent"), to Tabor *et al.* (hereinafter "Tabor"), assigned to President & Fellow [sic; Fellows] of Harvard College, Cambridge, Mass.

As noted in the cross-reference section of its specification, the above-captioned application is a continuation of U.S. Application No. 08/576,759, filed December 21, 1995, which is a continuation of U.S. Application No. 08/537,397, filed October 2, 1995, which is a continuation-in-part of U.S. Application No. 08/525,057, filed September 8, 1995. The '365 patent issued from U.S. Application No. 08/337,615 ("Tabor '615"), filed November 10, 1994,

which is a continuation-in-part of U.S. Application No. 08/324,437 ("Tabor '437"), filed October 17, 1994. Thus, the earliest possible effective filing date of the present application (September 8, 1995) is more than three months after the latest possible effective filing date of the '365 patent (November 10, 1994).

Therefore, in accordance with the requirements of 37 C.F.R. § 1.608(b), Applicant provides the following evidence demonstrating that Applicant is *prima facie* entitled to a judgment relative to patentee, and an explanation stating with particularity the basis upon which Applicant is *prima facie* entitled to the judgment.

I. Applicant is Prima Facie Entitled to Judgement Based on Priority of Invention

In order to establish that the Applicant is *prima facie* entitled to judgement, he can establish either an actual reduction to practice prior to Tabor's effective filing date or conception of the invention prior to Tabor's effective filing date coupled with diligence from just prior to Tabor's effective filing date until a later reduction to practice. Applicant demonstrates with the attached evidence that he actually reduced the invention to practice prior to October 17, 1994, the filing date of Tabor '437.[1]

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[1] Applicant notes that there are significant differences in disclosure between Tabor '437 and Tabor '615. By establishing an actual reduction to practice prior to the filing date of Tabor '437, Applicant does not imply that any claims of the '365 patent are entitled to priority from Tabor '437 under 35 U.S.C. § 120. Applicant expressly reserves his right to attack benefit of any Tabor claim from Tabor '437, e.g., under 37 C.F.R. § 1.633(g).

Applicant also reserves the right to establish priority based on other evidence, such as prior conception coupled with diligence.

A. Applicant Has Demonstrated Conception of His Invention Prior to October 17, 1994

Submitted herewith is a Declaration of the Applicant, Deb K. Chatterjee. This Declaration states that Deb Chatterjee conceived his invention before October 17, 1994. (Chatterjee Declaration at 2.) In order to substantiate this statement, a significant amount of evidence is submitted herewith, including Exhibits A-J attached to the Chatterjee Declaration, as well as corroborating Declarations (and associated exhibits) by Elizabeth Flynn, Adam Goldstein, A. John Hughes, Jr., Roger Lasken, Mary Longo, Brian Schmidt and Kalavathy Sitaraman. Exhibit C to the Chatterjee Declaration is a page from Deb Chatterjee's notebook describing a plan for making DNA polymerase mutations. The mutant polymerases were to be made by substituting amino acid residues of T7 polymerase for corresponding amino acids in a polymerase to be mutated. (*Id.* at 3-4.) Certain amino acids were identified in Chatterjee Exhibit C as important and thus candidates for substitution. Among these are Phe_{762} of Klenow fragment, which corresponds to Phe_{667} of *Taq* polymerase and Phe_{570} of T5 polymerase. (*Id.* at 3-4.) The corresponding amino acids in *Taq* and T5 polymerases were also identified in Chatterjee Exhibit C. Deb Chatterjee indicated in his Declaration that "this notebook page is dated prior to October 17, 1994, and was recorded prior to October 17, 1994." (*Id.* at 3.) Therefore, Deb Chatterjee had a definite and permanent idea of constructing mutant DNA polymerases comprising a substitution of Tyr for Phe at a position in the polymerase corresponding to Phe_{570} of wild-type T5 polymerase prior to October 17, 1994.

Another copy of another page from Deb Chatterjee's notebook is attached to the Chatterjee Declaration as Exhibit D. As Chatterjee avers in his declaration, this notebook page is dated prior to October 17, 1994, was recorded prior to October 17, 1994, and was based on

experiments conducted before October 17, 1994. This notebook page describes a 33 nucleotide oligonucleotide sequence that was used to produce mutant "F667Y," a mutant *Taq* DNA polymerase with polymerase activity and non-discrimination properties. Mutant "F667Y" is a single amino acid substitution in *Taq* DNA polymerase converting Phe_{667} to Tyr_{667}. This position corresponds to position 762 of *E. coli* polymerase I. Chatterjee Declaration at 4.

As stated in the Chatterjee Declaration, this oligonucleotide was identified as "Sequence 2680," and contains a created *Ase*I restriction site in addition to the nucleotide substitution, which results in mutant F667Y. These features can be observed directly on the sequence. The ATTAAT *Ase*I restriction site is noted on the sequence. In addition, the sequence GTA, which is the complement of the Tyr codon TAC, can be observed immediately adjacent to the *Ase*I restriction site. Since the full-length nucleotide and amino acid sequence of the wild-type *Taq* DNA polymerase gene and protein were known, *see supra*, the identification of this single amino acid substitution and the sequence of the synthetic oligonucleotide encoding this substitution also provides the full-length sequences for the F667Y mutant. Chatterjee Declaration at 4.

Deb Chatterjee ordered Sequence 2680 from Ms. Flora Lichaa, a fellow employee of Life Technologies, Inc., prior to October 17, 1994, and I received the synthesized oligonucleotide from Ms. Lichaa prior to October 17, 1994. *Id.* at 4-5.

As of October 17, 1994, it was well known in the art how to construct a DNA sequence coding for a structural gene containing a single amino acid substitution. *See, e.g.*, Sambrook *et al.*, *Current Protocols in Molecular Biology*, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press (1987), in a chapter entitled "Oligonucleotide-Directed Mutagenesis without Phenotypic Selection." Moreover, the full-length sequence of the wild-type *Taq* polymerase gene was published and known to Deb Chatterjee at that time. (Chatterjee Declaration at 3, and

Exhibit B thereto.) Therefore, prior to October 17, 1994, Deb Chatterjee had a definite and permanent idea of the complete and operative invention, *i.e.*, a mutant *Taq* DNA polymerase with polymerase activity and non-discrimination properties comprising a phenylalanine to tyrosine substitution at position 667, including its full-length sequence.

This definite and permanent idea of the mutant polymerase was communicated to others prior to October 17, 1994. Specifically, this idea was communicated to at least Harini Shandilya, Gary Gerard, and Flora Lichaa prior to October 17, 1994.

In a Declaration filed in parent U.S. Appl. No. 08/576,759, a copy of which is submitted herewith, Ms. Harini Shandilya indicated that she has worked in close physical proximity to Deb Chatterjee, and was asked periodically to witness his notebook. (Shandilya Declaration at 1-2.) Ms. Shandilya also indicates that she read, understood, and witnessed a page from Deb Chatterjee's notebook, which is attached to her Declaration as Exhibit A, prior to October 17, 1994. (*Id.* at 2-3.) This notebook page relates to a research plan to make *Taq* DNA polymerase mutants and refers to a distinctive difference between T7 polymerase and *Taq* polymerase to be the presence of a Tyr in lieu of a Phe at a position corresponding to Phe_{762} of *E. coli* Polymerase I (Klenow fragment). From this information, Harini Shandilya concludes that this notebook page teaches a plan for making, *inter alia*, a *Taq* DNA polymerase mutant at a position corresponding to the Phe_{762} of *E. coli* Polymerase I.[2] (*Id.* at 3.)

Exhibit B of the Shandilya Declaration is another notebook page recorded by Deb Chatterjee that was witnessed by Harini Shandilya prior to October 17, 1994. (*Id.* at 3.) This notebook page specifically identifies *Taq* polymerase mutant F667Y. (*Id.*) As described in the

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[2] This corresponds to position 667 of the *Taq* polymerase. (*See* Specification at 8.)

Shandilya Declaration at 3, the nomenclature "F667Y" refers to a substitution of Tyr (Y) for Phe (F) at position 667 of the *Taq* polymerase. Since the content of these notebook pages was read and understood by Harini Shandilya prior to October 17, 1994, the concept of this mutation was communicated to Harini Shandilya prior to October 17, 1994.

As discussed above and in the Chatterjee Delcaration, Ms. Flora Lichaa synthesized an oligonucleotide for the F667Y *Taq* polymerase mutagenesis experiment. A Declaration executed by Ms. Flora Lichaa was filed in parent U.S. Appl. No. 08/576,759, a copy of which is submitted herewith. Exhibit A of this Declaration is a Request for Oligonucleotide Synthesis that Flora Lichaa received from Deb Chatterjee prior to October 17, 1994. (Lichaa Declaration at 2.) This Request refers to an oligonucleotide to be synthesized for use in a "F667Y mutation of Taq." (*Id.*) As discussed *supra*, the nomenclature "F667Y" refers to a substitution of Tyr (Y) for Phe (F) at position 667 of the *Taq* polymerase. Therefore, the concept of this mutation was also communicated to Flora Lichaa prior to October 17, 1994.

A Declaration executed by Dr. Gary Gerard was also filed in parent U.S. Appl. No. 08/576,759, a copy of which is submitted herewith. Dr. Gerard's Declaration refers to a summary of information that he received from Deb Chatterjee prior to October 17, 1994, relating to the mutant DNA polymerase research project. (Gerard Declaration at 1-2.) A copy of Molecular Biology R&D Monthly Status Report prepared from this summary, with confidential information masked, is attached to the Gerard Declaration as Exhibit A. This summary refers to a plan for constructing Tyr to Phe mutations within the O-helix of *Taq* polymerase, T5 polymerase, and *E. coli* polymerase I. (Gerard Declaration at 1-2.) Phe_{667} of *Taq* polymerase is located within the O-helix. (*See* Chatterjee Declaration Exhibit C (*E. coli* polymerase I Phe_{762} is in the O-helix, and

this residue corresponds to Phe_{667} of *Taq* polymerase, *see supra*).) Therefore, information concerning these mutations was communicated to Gary Gerard before October 17, 1994.

In light of this evidence, Applicant submits that he has clearly demonstrated conception of his invention prior to October 17, 1994, and corroboration of that conception by communicating it to others prior to October 17, 1994.

B. Applicant Has Demonstrated an Actual Reduction to Practice of His Invention Prior to October 17, 1994

After conceiving of his invention, the Applicant proceeded to reduce his invention to practice. Applicant submits herewith the Declarations of Deb K. Chatterjee and several corroborating witnesses to establish, *inter alia*, an actual reduction to practice of at least one embodiment within the count prior to October 17, 1994. This evidence relates to the construction of a recombinant DNA molecule encoding a mutant F667Y *Taq* polymerase and the expression of the mutant F667Y *Taq* polymerase coupled with its testing for DNA polymerase activity.[3]

1. Construction of a Recombinant DNA Molecule Encoding a Mutant F667Y Taq Polymerase

Submitted herewith is a Declaration of Flora Lichaa. In this Declaration, Ms. Lichaa testifies that she constructed Oligonucleotide 2680 prior to October 17, 1994. (Lichaa Declaration at 2.) This oligonucleotide was synthesized for Deb Chatterjee for the stated purpose

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[3] As discussed *supra*, the position of this mutation corresponds to position 570 of wild-type T5 polymerase, and is within the scope of the proposed count.

of making a F667Y mutation in *Taq* DNA polymerase. This oligonucleotide has the following sequence:

GTA GAG GAC CCC GTA ATT AAT GGT CTT GGC CGC.

(Lichaa Declaration at 2, Exhibit A). This oligonucleotide encodes a Tyr at position 667 in place of Phe and also includes a created *Ase*I restriction site. (*See* Chatterjee Declaration at 4.)[4]

After Deb Chatterjee received this oligonucleotide he used it to perform oligonucleotide-directed mutagenesis on the *Taq* polymerase gene, and obtained at least one clone containing the oligonucleotide-derived sequence inserted into the *Taq* polymerase gene. (Chatterjee Declaration at 5-6, Exhibits E-F.)

The identity of this clone was confirmed in two different experiments. First, the clones derived from the mutagenesis were assayed for the presence of an additional *Ase*I restriction site derived from the synthetic oligonucleotide. The presence of this restriction site indicates that nucleotide sequences derived from the synthetic oligonucleotide were incorporated into the clone. The results of this experiment are depicted in Exhibits G and H to the Chatterjee Declaration, which contains a photocopy of the original data. These data indicate, as interpreted by Chatterjee and others, that at least one clone contains the additional *Ase*I restriction site. (*See* Chatterjee Declaration at 5-6.) Second, an *Ngo*AIV - *Xba*I restriction fragment of this clone was inserted into an inducible expression vector, the expression vector was transformed into bacteria, expression was induced, and the resulting culture was assayed and found to contain thermostable polymerase activity. (Chatterjee Declaration at 6-8, and Exhibits I and J.) Prior to transformation, the presence of the additional *Ase*I restriction site was confirmed in the

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[4] These changes can be confirmed by comparison to the DNA sequence of native *Taq* polymerase described in Lawyer *et al.*, *J. Biol. Chem. 264(11)*: 6427-6437 (1989) (Exhibit B to Chatterjee Declaration). *See infra*.

expression vector. (*Id.* at 6.) Thus, the expression vector, containing the additional *Ase*I restriction site, codes for thermostable DNA polymerase activity.

Since both the *Ase*I restriction site and the Phe to Tyr mutation at position 667 are derived from the oligonucleotide, the presence of this restriction site in the expression vector coupled with the actual expression of thermostable DNA polymerase activity is convincing evidence that the original clone contains a coding sequence for a mutant *Taq* polymerase containing the Phe to Tyr mutation at position 667. Thus, Applicant has demonstrated that a DNA molecule comprising this mutation, falling within the scope of the count, was constructed prior to October 17, 1994. The construction and cloning of this molecule constitutes an actual reduction to practice of this invention.

In constructing the expression vector containing DNA from the mutagenesis experiment, Applicant has reduced a second embodiment of his invention to practice. As discussed *supra*, this expression vector also contains the additional *Ase*I restriction site and directs the expression of thermostable DNA polymerase activity. Therefore, the expression vector also contains a coding sequence for a mutant *Taq* polymerase containing the Phe to Tyr mutation at position 667. Since this expression vector was constructed and tested for expression of polymerase activity prior to October 17, 1994, Applicant has demonstrated that a second DNA molecule falling within the scope of the count was constructed prior to October 17, 1994.

This actual reduction to practice has also been corroborated by non-inventors. *See Hahn v. Wong*, 13 U.S.P.Q.2d 1313, 1317 (Fed. Cir. 1989), *quoting Reese v. Hurst v. Wiewiorski*, 211 U.S.P.Q. 936, 940 (C.C.P.A. 1981) ("The inventor, however, must provide independent corroborating evidence in addition to his own statements and documents. Such evidence 'may consist of testimony of a witness, other than an inventor, to the actual reduction to practice or it

may consist of evidence of surrounding facts and circumstances independent of information received from the inventor.'") (citations omitted). Evidence relied upon for corroboration includes the testimony of Flora Lichaa regarding the synthesis of an oligonucleotide for the *Taq* F667Y mutagenesis experiment, the testimony of Adam Goldstein relating to the testing of host cells transformed with DNA molecules containing this mutant *Taq* polymerase gene for thermostable polymerase activity, the testimony of Harini Shandilya relating to her knowledge of the *Taq* polymerase F667Y mutagenesis experiments, and the original data in Deb Chatterjee's notebook as interpreted with particularity in the Declarations of Harini Shandilya, Adam Goldstein, and Deb Chatterjee.

As discussed *supra,* Flora Lichaa, a non-inventor, testified that she synthesized a synthetic oligonucleotide for Deb Chatterjee to be used to construct a F667Y mutation of the *Taq* polymerase prior to October 17, 1994. (Lichaa Declaration at 2.) While Deb Chatterjee has testified that this oligonucleotide contains a created *Ase*I restriction site and a nucleotide substitution resulting in the Phe to Tyr substitution at position 667 (Chatterjee Declaration at 4), this testimony can be confirmed based on information independent of the inventor.

First, in her Declaration, Flora Lichaa testifies to the exact sequence of the oligonucleotide she synthesized. (Lichaa Declaration at 2, Exhibit A) Moreover, the nucleotide sequence of the wild-type *Taq* polymerase gene has been published in Lawyer *et al.*, *J. Biol. Chem.*264: 6427-6437 (1989) (Exhibit B to the Chatterjee Declaration). By comparing the oligonucleotide sequence to the published nucleotide sequence of the *Taq* polymerase gene surrounding residue 667, it is possible to verify whether the oligonucleotide actually encodes the substitution alleged by Deb Chatterjee and a newly created *Ase*I restriction site. Both Harini Shandilya and Gary Gerard have performed this comparison and confirmed that the

oligonucleotide synthesized by Flora Lichaa is complementary to the nucleotide sequence reported in the Lawyer article with changes corresponding to the creation of the *Ase*I restriction site and conversion of a codon from Phe to Tyr. (Shandilya Declaration at 5; Gerard Declaration at 2.)

After receiving this oligonucleotide from Flora Lichaa, Deb Chatterjee performed an oligonucleotide-directed mutagenesis experiment prior to October 17, 1994. (Chatterjee Declaration at 5-6.) This testimony is corroborated by the testimony of Adam Goldstein, who testifies that he received cell culture samples from Deb Chatterjee transformed with the resulting mutant polymerase gene prior to October 17, 1994. (Goldstein Declaration at 2.) This cell culture was identified in Adam Goldstein's notebook as being from "Deb's clone pg. 174, Book 3573." (*Id.* at Exhibit 1.) Therefore, this testimony is independent evidence that the clone identified in Deb Chatterjee's notebook 3573 on page 174 (Chatterjee Declaration, Exhibit I) was constructed prior to October 17, 1994. Adam Goldstein then assayed these cultures and found that they contained thermostable DNA polymerase activity. (Goldstein Declaration at 2.) Thus, this testimony confirms that Deb Chatterjee actually constructed DNA molecules comprising a coding sequence for a mutant *Taq* polymerase containing the Phe to Tyr mutation at position 667.

This testimony of Deb Chatterjee is also corroborated by the testimony of Harini Shandilya. In her Declaration, Ms. Shandilya testified that during the time period Deb Chatterjee has alleged to have performed these mutagenesis experiments, she worked in close physical proximity to him, saw him performing molecular biology experiments, witnessed his laboratory notebooks, and confirmed that the general nature of the experiments she saw him perform is consistent with the notebook entries. (Shandilya Declaration at 2.) Specifically, Harini

Shandilya witnessed pages in Deb Chatterjee's notebook describing the mutagenesis of the *Taq* polymerase gene at position 667 (F667Y). (*Id.* at 2-3.)

This testimony is further corroborated by the actual data incorporated into Deb Chatterjee's notebook. *See Berges v. Gottstein*, 205 U.S.P.Q. 691, 694-95 (C.C.P.A. 1980) (NMR and IR spectra obtained by the inventor can be used as corroborating evidence). *Cf. Hahn*, 13 U.S.P.Q.2d at 1318 ("facts independent of the inventor's own assertions were needed to interpret the graphs.") Exhibits G and H to the Chatterjee Declaration are pages from Deb Chatterjee's notebook describing the testing of the original clones obtained from the oligonucleotide-directed mutagenesis experiment for the presence of the additional *Ase*I restriction site. These notebook pages also contain original data. Photocopies of the original data appear in Exhibits G and H.

The original data corresponding to these photocopies have been reviewed and interpreted by Harini Shandilya. Based on her independent review, she testified that the original data indicate that an additional *Ase*I restriction site has been incorporated into clone 8 described in Exhibit H. (Shandilya Declaration at 4.) DNA from this clone was inserted into an expression vector, used to transform bacteria, the expression vector was induced, and the culture was given to Adam Goldstein before October 17, 1994. This testimony confirms that these original data teach the incorporation of the oligonucleotide sequence, including the F667Y mutation, into the *Taq* polymerase gene of clone 8.

Based on this testimony and evidence, Applicant contends that the construction of a DNA molecule comprising a coding sequence for the F667Y *Taq* mutant protein has been established. Moreover, the construction of this DNA molecule has been corroborated by sufficient evidence independent of the inventor.

2. *Expression of a Mutant F667Y Taq Polymerase*

Applicant also submits testimony concerning the actual expression of a mutant F667Y *Taq* polymerase. Adam Goldstein testifies that he received a cell culture sample transformed with DNA encoding a thermostable mutant *Taq* polymerase where amino acid 667 was changed from Phe to Tyr prior to October 17, 1994. (Goldstein Declaration at 2.) This cell culture was identified in Adam Goldstein's notebook as being from "Deb's clone pg. 174, Book 3573." (*Id.* at Exhibit 1.) Therefore, this testimony is independent evidence that the clone identified in Deb Chatterjee's notebook 3575 on page 174 (Chatterjee Declaration, Exhibit H) was constructed prior to October 17, 1994.

Adam Goldstein also testifies that he has independently reviewed the original data incorporated into Deb Chatterjee's notebook 3575, page 174 to confirm the structure of the recombinant clone. (Goldstein Declaration at 3-4.) Based on this independent review, he has concluded that the clone he received from Deb Chatterjee contains an additional *Ase*I restriction site. (*Id.*) This additional *Ase*I restriction site is derived from the oligonucleotide used for the mutagenesis experiment. *See supra.* Therefore, this clone would also contain the sequence encoding the F667Y mutation, which is also derived from the oligonucleotide.

These cell culture samples received from Deb Chatterjee samples were tested by Adam Goldstein for thermostable polymerase activity prior to October 17, 1994. (*Id.*) Adam Goldstein testifies that thermostable polymerase activity was observed. (*Id.*) Since any thermostable polymerase activity would be derived from the expression of the mutant F667Y *Taq* DNA polymerase gene, this experimental result provides proof that the mutant F667Y *Taq* DNA polymerase was expressed and shown to have polymerase activity prior to October 17, 1994.

Since the testimony of Flora Lichaa, Adam Goldstein, Gary Gerard and Harini Shandilya is derived from noninventors, it need not be further corroborated to establish an actual reduction to practice. *See Holmwood v. Balasubramanyan Sugavanam*, 20 U.S.P.Q.2d 1712, 1715 (Fed. Cir. 1991) ("Only an inventor's testimony needs corroboration.").

In light of this evidence, Applicant contends that he has established at least two actual reduction to practices of his invention prior to October 17, 1994: (1) the construction of a DNA molecule comprising a coding sequence for the F667Y mutant of the *Taq* polymerase; and (2) the expression and testing of the F667Y mutant *Taq* polymerase for thermostable polymerase activity. Therefore, Applicant is *prima facie* entitled to judgement based on priority of invention.

D. Applicant Was Diligent Between the Conception and Reduction to Practice, and the Application Filing Date

Following the conception of the claimed invention, Applicant supervised a diligent effort to reduce this invention to practice. As discussed above, this diligent effort began before October 17, 1994, and included the cloning and expression of the mutant *Taq* DNA polymerase. While the *Taq* DNA polymerase had been cloned and expressed prior to October 17, 1994, diligent activity involving *Tne*, *Tma*, and *Taq* polymerases continued after October 17, 1994, until the filing of the great-grandparent application on September 8, 1995. *See* Chatterjee Declaration at 8.

This diligent effort included several different activities. As one step, the properties of several different wild-type DNA polymerases were characterized including T5, *Thermotoga neapolitana* (*Tne*), *Thermotoga maritima* (*Tma*), *Thermus flavus* (*Tfl*), *Thermus thermophilus* (*Tth*) and the Klenow fragment of *Escherichia coli* DNA polymerase I. A second step involved

the cloning and expression of the wild-type genes encoding these polymerases. A third step involved introducing mutations into the genes encoding the polymerases. A fourth step involved transforming host cells with the mutant polymerase genes, expressing the mutant polymerases, and characterizing the biological activity of the mutant polymerases. *See* Chatterjee Declaration at 9.

Mutations introduced in the DNA polymerases included mutations in the 5'-3' exonuclease domain to reduce or eliminate 5'-3' exonuclease activity, mutations in the 3'-5' exonuclease domain to reduce or eliminate 3'-5' exonuclease activity, in addition to mutations in the O-helix. More specifically, the mutant polymerase includes a mutation in a tyrosine residue at an amino acid position corresponding to position 667 of the *Taq polymerase* of the wild-type DNA polymerase. This position is located in the O-helix of Pol I-type DNA polymerases. The specific position of the wild-type DNA polymerase is, for example: *E. coli* bacteriophage T5 (T5) DNA polymerase I residue 570, *E. coli* (Klenow fragment) DNA polymerase I residue 762, *E. coli* bacteriophage T7 (T7) DNA polymerase I residue 526, *Thermotoga neopolitana* (*Tne*) DNA polymerase residue 67 of the sequence depicted in Figure 4 of the specification, *Thermotoga maritima* (*Tma*) DNA polymerase residue 730, *Streptococcus pneumoniae* DNA polymerase residue 711, *Thermus flavus* (*Tfl*) DNA polymerase residue 666, *Thermus thermophilus* DNA polymerase residue 669, *Deinococcus radiodurans* DNA polymerase residue 747, *Bacillus caldotenax* DNA polymerase residue 711, mycobacteriophage L5 DNA polymerase residue 438, *E. coli* bacteriophage SP01 DNA polymerase 692 and *E. coli* bacteriophage SP02 DNA polymerase 447. As described in the specification, the polymerase can be modified to reduce 3' to 5' exonuclease activity and 5' to 3' exonuclease activity in addition to the mutation in the O-helix. *See* Chatterjee Declaration at 9-10.

During the period from just prior to October 17, 1994, until September 8, 1995, individuals under the supervision of Applicant worked on the cloning, expression, and characterization of wild-type and mutant DNA polymerases. *See* Chattjeree Declaration at 10. These individuals included Carolyn Combs, Barbara Flynn, Elizabeth Flynn, Adam Goldstein, A. John Hughes, Jr., Roger Lasken, Flora Lichaa, Mary Longo, Avani Patel, Brian Schmidt, Harini Shandilya and Kalavathy Sitaraman. *See Id.* As part of the responsibilities of Applicant in the laboratories of the assignee of the present application, Applicant would discuss experiments to be performed and would be kept informed of the results of these experiments on a regular basis. *See Id.* For example, from October 1994 until September 1995, it was the practice of Applicant to conduct weekly laboratory meetings. *See Id.* All of the individuals listed above typically attended these weekly laboratory meeting, which were typically held on Monday mornings. During this time period, recently obtained results relating to the DNA polymerase project were often discussed. *See Id.*

As Applicant states in his Declaration, he has reviewed the laboratory notebooks of the individuals working on the project. *See Id.* Based on these laboratory notebook records and his recollection, Applicant states that certain activities relating to the DNA polymerase project took place under the supervision of Applicant from October 16, 1994, until September 8, 1995. These activities are described in detail in the declarations and attachments thereto by Elizabeth Flynn, Adam Goldstein, A. John Hughes, Jr., Roger Lasken, Mary Longo, Brian Schmidt and Kalavathy Sitaraman which are filed concurrently herewith, and which are incorporated by reference herein and made a part hereof in their entireties. Since the testimony of Elizabeth Flynn, Adam Goldstein, A. John Hughes, Jr., Roger Lasken, Mary Longo, Brian Schmidt and Kalavathy Sitaraman is derived from a noninventor, it need not be further corroborated to establish

diligence or an actual reduction to practice. *See Holmwood v. Balasubramanyan Sugavanam,* 20 U.S.P.Q.2d 1712, 1715 (Fed. Cir. 1991) ("Only an inventor's testimony needs corroboration.").

II. Conclusion

For at least the reasons presented above, and in light of the attached evidence and remarks, Applicant respectfully requests that an interference be expeditiously declared between the present application and U.S. Patent No. 5,614,365.

Respectfully submitted,

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